Identification of Novel Genes Differentially Expressed in Omental Fat of Obese Subjects and Obese Type 2 Diabetic Patients

Helena Corominola,1 Laura J. Conner,2 Lisa S. Beavers,2 Robert A. Gadski,2 Dwayne Johnson,2 Jose F. Caro,2 and Ronit Rafaeloff-Phail2

Obesity is associated with an increased risk for developing type 2 diabetes, insulin resistance, hypertension, dyslipidemia, cardiovascular disease, respiratory dysfunction, and certain forms of cancer. Insulin resistance in many type 2 diabetic patients is the result of increased visceral adiposity. To identify novel genes implicated in type 2 diabetes and/or obesity and to elucidate the molecular mechanisms underlying both diseases, we analyzed gene expression in omental fat from lean and obese nondiabetic subjects and obese type 2 diabetic patients using mRNA differential display and subtracted library techniques. After screening over 13,800 subtracted cDNA clones and 6,912 cDNA amplification products, we identified 2,078 cDNAs that showed potential differential expression in the omental fat of lean versus obese nondiabetic subjects versus obese type 2 diabetic patients. Data analysis showed that 70.7% of these clones corresponded to unknown genes (26.7% matched express sequence tags [ESTs]) and 29.3% corresponded to known genes. Reverse Northern and classic Northern analyses further confirmed that the expression of five of these cDNA clones was elevated in obese nondiabetic subjects and obese type 2 diabetic patients. Four candidate genes were further evaluated for tissue distribution, which showed expression primarily in adipose and skeletal muscle tissue, and chromosomal localization. We concluded that both mRNA differential display and subtracted cDNA libraries are powerful tools for identifying novel genes implicated in the pathogenesis of obesity and type 2 diabetes. Diabetes 50:2822–2830, 2001
Differential display analysis

Subtracted libraries

Data are means ± SE. ND, not determined.

Diabetes and obese diabetic minus obese nondiabetic omental adipose tissue by suppression PCR (Clontech). Then ~9,200 independent clones from obese nondiabetic minus obese type 2 diabetic and 4,600 from obese diabetic minus obese nondiabetic were randomly picked into 96-well plates and arrayed in duplicate onto charged nylon membranes (Amersham, Piscataway, NJ) using a PBA Flexys robot (Genomic Solutions, Ann Arbor, MI). Clones were identified by differential hybridization to 32P-labeled cDNA probes generated by reverse transcription of 10 μg of total RNA (isolated from the same patients used to construct the library) by oligo-dT priming, using the Superscript Preamplification Kit (Gibco/BRL) (Fig. 3). Image analysis was performed using a Personal FX Phosphorimager and AIB (Imaging Research, ON, Canada) imaging software for colony arrays.

Plasmid cDNA of potentially differentially expressed clones was alkaline denatured, rearranged in duplicate, and probed as above. Putative differentially expressed genes were isolated.

This gel is a representative of similar gels from which other candidate genes were isolated.

**FIG. 1.** Gel analysis of differential display of human omental fat RNA. Total RNA was isolated from omental fat of four lean nondiabetic (L), four obese nondiabetic (O), and four obese type 2 diabetic patients (D) and treated with DNase. Duplicate reverse transcriptase–PCR reactions were performed, as described in RESEARCH DESIGN AND METHODS. Radiolabeled products were resolved side by side on a 6% sequencing gel and analyzed by autoradiography. The arrow indicates one band, DD19, which was differentially expressed in omental fat of obese type 2 diabetic patients relative to lean and obese nondiabetic patients.

**TABLE 1**
Clinical characteristics of human adipose tissue donors

<table>
<thead>
<tr>
<th>n</th>
<th>Age (years)</th>
<th>BMI (kg/m²)</th>
<th>Fasting plasma glucose (mg/dl)</th>
<th>Glucose 120 min (mg/dl)</th>
<th>Fasting plasma insulin (μU/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lean</td>
<td>4</td>
<td>36.5 ± 9.5</td>
<td>22.7 ± 2.0</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Obese nondiabetic</td>
<td>4</td>
<td>32.9 ± 14.9</td>
<td>45.7 ± 5.0</td>
<td>93.8 ± 6.4</td>
<td>132.5 ± 12.5</td>
</tr>
<tr>
<td>Obese type 2 diabetic</td>
<td>4</td>
<td>39.7 ± 5.7</td>
<td>56.3 ± 1.8</td>
<td>150 ± 29.6</td>
<td>236 ± 33.9</td>
</tr>
</tbody>
</table>

RNA isolation. Total RNA was extracted from human tissue by homogenization using a Polytron LS 10-35 (Kinematica, Cincinnati, OH) followed by guanidinium isothiocyanate-phenol chloroform extraction (8). The quality of the RNA was verified by ethidium bromide staining of rRNA bands on a gel. To remove contaminating DNA from the RNA preparations, samples were incubated with Rnase-free DNase I (MessageClean Kit; Genhunter, Beverly, MA). Samples were then reamplified into DH5a (Gibco/BRL). For each clone, 10 colonies were picked and cDNA was synthesized from total RNA using SMART cDNA Synthesis Kit (Clontech, Palo Alto, CA), and subtracted cDNA libraries were generated from obese nondiabetic minus obese type 2 adipose tissue.

Total RNA was isolated from omental fat of four lean nondiabetic, four obese nondiabetic, and four obese type 2 diabetic patients by oligo-dT priming using the Superscript Preamplification Kit (Gibco/BRL) (Fig. 3). Image analysis was performed using a Personal FX Phosphorimager and AIB (Imaging Research, ON, Canada) imaging software for colony arrays.

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TABLE 2
Sequences corresponding to known genes identified by differential display of mRNA and subtractive libraries

<table>
<thead>
<tr>
<th>cDNA ID</th>
<th>Differential display</th>
<th>Subtractive libraries</th>
</tr>
</thead>
<tbody>
<tr>
<td>DD7</td>
<td>Plasma retinol binding protein</td>
<td>Decorin</td>
</tr>
<tr>
<td>DD8</td>
<td>Transcription factor ISGF-3</td>
<td>Glutamine Synthetase</td>
</tr>
<tr>
<td>DD16</td>
<td>Nuclear-phosphoprotein 130</td>
<td>G36 antigen-glycoprotein</td>
</tr>
<tr>
<td>DD17</td>
<td>Glucocorticoid receptor repression factor 1</td>
<td>GPIIIb/GP IV</td>
</tr>
<tr>
<td>DD22</td>
<td>Ornithine decarboxylase antizyme</td>
<td>Human laminin B2 chain</td>
</tr>
<tr>
<td>DD34</td>
<td>ATP diphosphohydrolase</td>
<td>Glycerol 3-phosphate alyctransferase</td>
</tr>
<tr>
<td>DD40</td>
<td>Complement C1 S</td>
<td>Complement C3 precursor</td>
</tr>
<tr>
<td>DD43</td>
<td>Complement C3 precursor</td>
<td>CD59 glycoprotein</td>
</tr>
</tbody>
</table>

RESULTS
Identification of differentially expressed human omental fat genes. To identify alterations in gene expression associated with obesity and type 2 diabetes using the mRNA differential display, we isolated total RNA from omental fat of four lean, four obese nondiabetic, and four obese type 2 diabetic patients. For each RNA, 96 combinations were probed with cDNA for β-actin. Data were quantified as densitometric units normalized per β-actin gene signal.

Chromosomal localization. Genomewide radiation hybrid analysis was performed with the Stanford Radiation Hybrid (RH) G3 panel (Research Genetics, Huntsville, AL). This panel contains 83 whole genome radiation hybrids formed by somatic cell hybrids containing portions of the human genome on a hamster background (11). The presence of the EST in the RH cell lines and three controls (Chinese hamster genomic DNA, human genomic DNA, and no DNA) was determined by PCR using gene-specific oligonucleotides. The PCR reaction reagents were 0.4 μmol/l of gene-specific primers and 1 × Platinum PCR SuperMix (Gibco-BRL). Each sample was amplified in duplicate and analyzed by agarose gel electrophoresis. Gels were scored for the presence or absence of positive PCR products at the expected size, and only products present in duplicate were scored as positive. Results were submitted to the RH mapping e-mail server at Stanford Human Genome Center (SHGC) for two-point maximum likelihood analysis. This allowed determination of logarithm of odds (LOD) scores for each EST versus the 954 SHGC framework markers.

TABLE 3
Nonredundant nucleic acid database BLASTN analysis of human omental cDNAs identified by differential display of mRNA and subtractive libraries

<table>
<thead>
<tr>
<th>Novel sequences</th>
<th>31 (79.5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No matches</td>
<td>15</td>
</tr>
<tr>
<td>Matches to ESTs</td>
<td>16</td>
</tr>
<tr>
<td>Known genes</td>
<td>8 (20.5)</td>
</tr>
</tbody>
</table>

Data are n (%)

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fragments were purified, reamplified, cloned, and sequenced as described in Research Design and Methods. After sequencing and comparison of the 51 cDNA fragments with Genbank and Lilly proprietary databases, 2 corresponded to mitochondrial genes, 10 did not reamplify, and 8 were identical to known genes. Of those eight known genes, it has been suggested that the complement C3 precursor may affect lipid metabolism and that plasma retinol-binding protein (RBP) and ornithine decarboxylase may be associated with type 2 diabetes (Table 2). The 31 remaining sequences were either highly homologous to public ESTs (51.6%) or were not contiguous with any

FIG. 2. Analysis of differential gene expression in omental adipose tissue from obese nondiabetic minus obese type 2 diabetic patients (left) and obese type 2 diabetic minus obese nondiabetic subjects (right). Analysis of differential gene expression on cDNA arrays identified potential regulated genes. Blots were hybridized with 32P-labeled first strand cDNA synthesized from 10 µg of total RNA isolated from obese nondiabetic and obese type 2 diabetic patients. The arrows mark candidate cDNAs preferentially amplified from omental fat of obese nondiabetic subjects relative to obese type 2 diabetic patients (2040, 3345).

FIG. 3. Reverse Northern blot analysis of differentially expressed cDNAs from obese nondiabetic minus obese type 2 diabetic library. Clones were amplified using either vector or gene-specific primers, run on a 1% agarose gel, and transferred to nylon membranes. Membranes were hybridized with oligo-dT–primed 32P-labeled cDNAs probes and analyzed using a Personal FX Phosphoimager and QuantityOne software. This figure represents the data of four independent experiments with similar results.
known genes (48.4%) (Table 3). In all the clones, the sequence of the primers used for the differential display was present. Interestingly, a large number of those clones had the same primer on both ends.

To generate the subtractive cDNA libraries, total RNA was isolated from omental adipose tissue from four obese nondiabetic and four obese type 2 diabetic female patients, and was used for cDNA synthesis and construction of the libraries. We randomly picked ~9,200 independent clones from obese nondiabetic minus obese diabetic and 4,600 from obese diabetic minus obese nondiabetic subjects from omental adipose cDNA libraries; these were arrayed in duplicate onto charged nylon membranes and hybridized with labeled probes derived from the same patients used to construct the libraries. This analysis indicated that the 13,800 clones included 2,039 distinct fragments corresponding to putative differentially expressed RNAs (Fig. 2). Plasmid cDNAs of these potentially differentially expressed clones were arrayed in duplicate, reprobed, sequenced, and subjected to BLAST analysis. As shown in Table 3, 600 (29.4%) of these clones corresponded to known genes. These included genes previously known to be differentially expressed in obesity and/or type 2 diabetes as well as genes that had not been previously recognized to be modulated in these diseases (Table 2). The remaining 1,439 (70.6%) sequences were classified as novel, representing either nearly exact matches with ESTs (539 clones) or previously unreported sequences (900 clones) (Table 3). Based on the sequence information and a differential expression of >1.5-fold, we selected 195 clones to be followed up and validated by reverse Northern analysis.

Confirmation of differential expression in obesity and type 2 diabetes. To confirm differential expression of the newly identified genes, 28 candidates from the differential display analysis and 195 from the subtractive libraries were selected and analyzed by reverse Northern analysis (four independent experiments). A total of 26 genes, 12 from the differential display and 14 from the subtractive libraries, displayed greater than threefold differential expression among lean nondiabetic, obese nondiabetic, and obese diabetic patients (Fig. 3). As a final validation for the differential expression of these candidate genes and to identify transcript size, we used the

![Image](image_url)
TABLE 4
Sequence information and expression levels of the candidate genes and leptin

cDNA identification  Sequence name  Sequence*  Expression level information

Subtractive libraries
2040  Novel  AJ318806  4.8 D/L  8.2 O/D
3345  Novel  AJ318807  3.5 D/L  1.8 D/O
3001  Decorin  BE045488  1.5 D/L  NSD
3038  EST  3304  CROC-1A DNA  NSD  NSD
3333  EST  BF593650  2.1 D/L  2.1 O/D
2024  Novel  AJ318808  2.1 D/L  NSD
3409  EST  AU144391  NSD  NSD
Leptin  8.2 D/L  1.71 D/O

Differential display
DD5  Novel  AJ318806  1.5 D/L  1.41 D/O
DD19  Novel  AJ318804  1.7 D/L  1.3 D/O
DD44  EST  AB018268  1.5 D/L  1.2 D/O

Expression levels of the candidate genes (displaying >1.5-fold differential expression by reverse Northern analysis) and leptin among lean (L), obese nondiabetic (O) and obese type 2 diabetic (D) patients determined by Northern blot analysis. *Sequence is Genbank accession or clone matches number. Leptin expression was increased by 8.2- and 1.7-fold in D patients vs. L and O subjects, respectively. NSD, no significant differences.

more reliable and accurate technique of classic Northern blot analysis. Pooled total RNA derived from omental fat of 7 lean nondiabetic, 17 obese nondiabetic, and 12 obese type 2 subjects was resolved on a 1% agarose/formaldehyde gel, transferred to a Nytran-plus membrane, and hybridized with the relevant radiolabeled cDNA. To test whether the gene expression pattern in the omental fat of the patients used in this study was representative of the general human population with obesity and type 2 diabetes, we performed a Northern blot analysis using leptin as a probe. Leptin mRNA levels were increased by 8.17- and 1.71-fold in type 2 diabetic patients versus lean and obese patients, respectively (Fig. 4A). These results concurred with those in a previous report (12), validating our subset of patients as representative of the human population presenting these diseases.

Of the 26 candidate genes, 13 showed a visible hybridization signal. The remaining 13 genes showed either no signal or a smear on the blot, despite prolonged exposure. As shown in Table 4, five novel genes shared a common pattern profile of increased expression in fat of type 2 diabetic patients and a more variable expression in obese nonobese subjects. In the fat of obese nondiabetic patients, the expression of these genes was either comparable to (3001, decorin) or lower (3345, DD5, DD19, DD44) than that in type 2 diabetic subjects, and either similar to (2024, DD5) or higher (3001, decorin, 3345, DD19, DD44) than expression levels in fat from lean nonobese subjects. Five genes did not show significant differences in their mRNA levels, and two genes (2040, 2024) were upregulated in obese nondiabetic patients when compared with lean nondiabetic and obese type 2 diabetic patients.

Based on the level of differential expression, four clones—2040 (Fig. 4B), 3345 (Fig. 4C), DD5 (Fig. 4D), and DD19 (Fig. 4E)—were chosen for tissue distribution analysis. None of these cDNAs showed homology with published sequences in public and proprietary databases. We chose not to pursue gene 2024, although it showed significant differential expression, because the results did not confirm the original findings from the subtracted library.

DD19 and 2040 were electronically extended using public and proprietary databases. DD19 electronic extension yielded a putative gene sequence with high homology to a published sequence (GenBank accession #AR062278) from patent U.S. 5843716, which encodes for a proline-rich membrane protein. Full-length cloning by PCR, using primers that matched the electronically extended sequences, confirmed that DD19 represented a partial cDNA of the published sequence. Electronic extension of 2040 followed by PCR yielded a longer fragment (4 kb) but did not match any known sequence; 3345 and DD5 did not extend electronically.

Tissue distribution. To analyze tissue distribution of the candidate genes (2040, 3345, DD5, and DD19), we performed Northern blot analysis using polyA + RNA or total RNA from human tissues. As expected, expression of all candidate genes was higher in adipose tissue than in other tissues. The transcript of 2040 gene was 10 kb in length and was uniquely expressed in adipose tissue (Fig. 5A), whereas the 3.6-kb transcript of 3345 was also detected at significant levels in total RNA from human placenta (Fig. 5B). A 2.6-kb transcript was identified for DD19, which was predominantly expressed in RNA from human adipose tissue (Fig. 5C) and human skeletal muscle (not shown). A lower expression of this gene was found in RNA of human heart (not shown). The DD5 gene, whose transcript was 4.4 kb in length, was highly expressed in RNA from human skeletal muscle (Fig. 5D) and adipose tissue (not shown).

Chromosomal localization. DD5, DD19, 2040, and 3345 genes were selected for PCR-based radiation hybrid analysis. All the candidate genes mapped on the RH panel were linked to Genethon meiotic linkage markers with a LOD score >6; their cytogenetic location was estimated by the genetic markers flanking the mapped genes. DD5 was mapped to the short arm of chromosome 6, close to marker AFM 203yg7 in the 6p21 region; DD19, to chromosome 2, approximately in the cytogenetic location 2q34–2q35; 3345, to chromosome 17, close to marker AFM 191h6, approximately in the cytogenetic location 17p11; and 2040, to chromosome 3, close to marker D3S1620, approximately in the cytogenetic location 3p25.
DISCUSSION

In the present study we applied mRNA differential display and subtractive cDNA library techniques to identify genes that are differentially expressed in omental adipose tissue of patients with type 2 diabetes and/or obesity. The need for this approach arose from the observation that the insulin resistance preceding type 2 diabetes can be acquired as a result of obesity, particularly upper-body obesity with the subsequent accumulation of visceral fat (4). Furthermore, several substances produced by adipocytes have been implicated in the etiology of insulin resistance, such as circulating free fatty acids as well as tumor necrosis factor-α (TNF-α) and leptin (5–7). Despite evidence suggesting an active role of visceral fat in the development of type 2 diabetes, to our knowledge this is the first study reporting the application of these techniques to identifying novel genes associated with obesity and type 2 diabetes in human omental adipose tissue. Using mRNA differential display and screening of subtracted cDNA libraries, we isolated 2,078 distinct fragments that seemed to be differentially expressed. Of those, 608 corresponded to known genes and 1,470 to novel sequences either homologous to ESTs in the public database or previously unreported sequences. Most of the known genes that we identified affect lipid metabolism or encode for proteins of the complement system, with some of these genes being involved in other cell functions.

Confirmation of gene expression is one of the crucial steps after mRNA differential display or subtracted libraries. A large number of false-positive bands may be present on differential display, and false-positive clones may be found in the subtractive libraries. A variety of methods to reduce false positives have been used in different laboratories. The most commonly used and most reliable technique is classic Northern blot analysis. However, Northern blot analysis requires relatively high quantities of RNA. This becomes problematic when dealing with difficult-to-obtain human tissue. For this reason, we initially used reverse Northern analysis, which requires less RNA, to eliminate most of the false-positive clones. For the most
promising clones, we reconfirmed the results using classic Northern blot analysis.

The complement C3 precursor gene was found by both differential display (DD43) and screening of the diabetic minus obese nondiabetic library (clone #3481) to be upregulated in type 2 diabetic patients. The interaction of complement C3 protein with factor B and adipins resulted from the cleavage of complement C3-generating acylation-stimulating protein (ASP) (13). ASP can actively stimulate triacylglycerol synthesis in human adipocytes and plays an essential role in the normal clearance and disposition of dietary fatty acids. Recently, a study reported that plasma levels of ASP were significantly higher in obese subjects compared with lean subjects (14), and mRNA levels of ASP have been found to be higher in visceral fat compared with abdominal subcutaneous adipose tissue in obese individuals (15). Taken together, these data may indicate an involvement of the ASP/adipin pathway in the pathogenesis of obesity and type 2 diabetes.

Fatty acid–binding protein (FABP)-2 was also upregulated 10-fold more in omental adipose tissue of obese nondiabetic versus type 2 diabetic patients in our study (clone #205, confirmed by reverse Northern analysis; data not shown). Genetic studies have suggested that FABP2 does not represent a major gene for type 2 diabetes, but does seem to be related to the metabolic insulin-resistance syndrome (16–18).

DD7, a gene in our study that was found to be upregulated in omental fat of type 2 diabetic patients, shows a high homology to plasma RBP. Human plasma RBP is encoded by a single gene and is known to be synthesized by the liver. The function of this protein is to deliver retinol from liver stores to peripheral tissues. In plasma, the RBP-retinol complex interacts with transthyretin, which prevents its loss by filtration through the kidney glomeruli (19). After delivering retinol to target cells, the RBP is rapidly cleared from the circulation by the kidney. One study reported elevated levels of RBP in type 2 diabetic subjects, particularly in those with higher nonfasting insulin levels (20). It is not clear what the significance of adipocyte-derived RBP is because the main site of synthesis is the liver, but it could merely be an indicator of metabolic control in these patients. Alternatively, because RBP plays an important physiological role in retinol transport and recycling, the expression of RBP in adipose tissue, a vitamin A target tissue, may reflect the extent of retinoid metabolism in type 2 diabetic patients.

In our search for novel genes associated with obesity and type 2 diabetes, we have also found differentially expressed genes encoding proteins involved in protein metabolism, such as glutamine synthetase, and genes encoding for structural proteins, such as decorin and laminin. We found that glutamine synthetase, an enzyme that forms glutamate from glutamine, was upregulated in omental fat from type 2 diabetic patients. To our knowledge, this is the first study to show increased levels of this enzyme in adipose tissue. It has been previously reported that glutamine synthetase activity and mRNA levels were elevated in muscle from streptozotocin-induced diabetic rats and were reversed by insulin administration (21). We could speculate that high levels of glutamine synthetase may contribute to the substantial alterations of glutamine and alanine metabolism observed in type 2 diabetes.

From our findings and previous reports, one might postulate a role for decorin and laminin in type 2 diabetes. Decorin and laminin are both structural proteins. It has been demonstrated that laminin and decorin levels are increased in hyperglycemia (22). In addition, induction of diabetes in rodents leads to an increase in kidney decorin levels (23). Further studies are required to clarify whether the increased levels of these proteins are a direct effect of the ambient high glucose or a primary defect in type 2 diabetes.

In this study, we identified four candidate genes truly modulated in omental adipose tissue of obese and type 2 diabetic patients. We studied their tissue distribution as well as their chromosomal localization. Database comparisons showed that three of these genes (DD19, 2040, and 3345) were represented as EST sequences and one (DD5) was a novel sequence not represented in the public databases. Expression of all the candidate genes was higher in adipose tissue than in other tissues, suggesting that their function is probably related to adipocyte metabolism. Two genes, DD5 and DD19, also showed a significant expression in muscle. The chromosomal localization studies showed that all of the candidate genes were mapped to chromosomal regions previously linked to the pathogenesis of obesity or type 2 diabetes, supporting an important role of our candidate genes in these diseases.

DD5 was mapped to the chromosomal region 6p21. Interestingly, TNF-α, which is considered a candidate gene for obesity, has also been localized to that region (24). TNF-α expression is increased in adipose tissue of both rodent models of obesity and obese humans, and a linkage between obesity and a marker near the TNF-α locus has been reported in Pima Indians (25). Furthermore, recent data suggest a key role for TNF-α in the insulin resistance of obesity and type 2 diabetes (26). Although highly speculative, the chromosomal proximity of DD5 and TNF-α may suggest a role of DD5 in insulin resistance, something that needs to be further investigated. The chromosomal mapping of 2040 to chromosome 3, approximately in the telomeric region 3p25, is also interesting. Peroxisome proliferator–activated receptor-γ (PPAR-γ) has been mapped to the same region 3p25 (27), within 1.5 Mb of a suitable polymorphic marker that could be used in linkage analysis to evaluate a potential contribution of PPAR-γ to lipid metabolism–related diseases (28). One might speculate that the location of 2040 in the same region could also indicate a contribution of 2040 to lipid metabolism. DD19 was mapped to chromosomal region 2q34–2q35. Significant linkage has been reported between chromosome 2, leptin levels, and fat mass (29). A major susceptibility locus for type 2 diabetes has been identified by linkage analysis on chromosome 2 in a Mexican-American population (30). Finally, 3345 has been mapped to chromosomal region 17p11. Recently, two loci on chromosome 17 have been linked to increased susceptibility to obesity in a Mexican-American population (31). GLUT4, the gene encoding the insulin-responsive glucose transporter, has also been mapped to chromosome 17 (32).
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The authors wish to express their appreciation to Drs. G.L. Dohn and W. Pores at Eastern Carolina University Medical Center for organizing the patients, gathering the clinical data, and collecting the adipose tissue samples.

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